METHOD AND APPARATUS FOR BACKSCATTER SPECTROSCOPY

The present invention relates to a method of determining of a physical feature of a medium, comprising:

- producing radiation with a light source;
- placing a probe on a sample of said medium, the probe comprising a first optical fiber having a first diameter, and at least a second optical fiber having a second diameter;
- sending light coming from the light source, through the first optical fiber;
- collecting first backscattered radiation through the first optical fiber and second backscattered radiation through the second optical fiber;
- producing a first signal based on the first backscattered radiation, and a second signal based on the second backscattered radiation;
- determine a measured differential backscatter signal as a function of wavelength using the first and second signals.

Such a method is known from Amelink et al [1]. There, a special device is used to determine particle sizes in superficial layers. The device is suitable for measuring particle sizes in for example an aqueous suspension with polystyrene spheres, but is not fitted to accurately measure particle sizes in living tissue. So, determining whether living tissue is normal or precancerous, by way of measuring particle sizes in living tissue is not very promising.

In Doornbos et al [2] the optical properties of human tissue are determined in vivo using a spectroscopic arrangement with ten optical fibers. One of the fibers is used to irradiate a sample, and nine other fibers collect the reflected light. By using a multitude of fibers to collect the reflected light, it is possible to calculate scattering and absorption coefficients of the sample. However, the method is not suitable for locally measuring the optical properties of the tissue. In particular, only mean values of the absorption coefficient of a relatively large part of the sample can be determined.

It is an object of the present invention to locally measure a physical feature, such as a concentration, of a substance in a medium.

The object is achieved by a method as described above, characterized by

- calculating the physical feature by curve fitting said measured differential backscatter signal to a backscatter function, in which the backscatter function is a function of an

average path-length travelled by detected scattered photons, wherein the average path-length is independent from an absorption coefficient of the medium, and from a scattering coefficient of the medium. Contrary to methods using diffusely scattered photons such as Doornbos et al [2], in the method according to the invention, the local absorption coefficient of the sample is measured in an absolute way, independent of the magnitude of the local scattering and absorption coefficients. This facilitates the measurement of absolute concentrations of absorbing molecules in a sample without requiring prior knowledge of the magnitude of the scattering and absorption coefficients of the medium.

In an embodiment, the average path-length is proportional to the first fiber diameter. This has as additional advantage that the average path-length and thereby the average penetration depth into the sample of the photons that contribute to the differential backscatter signal can be controlled by choosing the fiber diameter. As a result, the sampling volume can be controlled by adjusting the fiber diameter. Hence, the fiberoptic probe can be engineered to match the relevant dimensions of the medium under investigation.

In a particular embodiment, the physical feature is a concentration of at least one substance in the medium.

The invention also relates to a device for determining a physical feature of a medium, comprising:

- a light source for producing radiation;
- a probe with at least a first and a second optical fiber, the first optical fiber having a first diameter and being arranged to deliver the radiation on a sample of said medium and to collect first backscattered radiation from said sample, the second optical fiber having a second diameter and being arranged to collect second backscattered radiation, wherein the second optical fiber is positioned alongside the first optical fiber;
- a spectrometer for producing a first signal based on the first backscattered radiation, and for producing a second signal based on the second backscattered radiation;
- a processor arranged to determine a measured differential backscatter signal as a function of wavelength using the first and second signals,

characterized in that the processor is arranged to calculate the physical feature by curve fitting the measured differential backscatter signal to a backscatter function, in which the backscatter function is a function of an average path-length travelled by detected

scattered photons, the average path-length being independent from an absorption coefficient of the medium, and from a scattering coefficient of the medium.

Furthermore, the invention relates to a computer program according to claim 8and to a data carrier according to claim 9.

In another aspect of the invention, the invention relates to a method of determining a physical feature of a medium, comprising:

- producing radiation with a light source;
- placing a probe on a sample of the medium, the probe comprising a first optical fiber having a first diameter, and at least a second optical fiber having a second diameter;
- sending light coming from the light source, through the first optical fiber;
- collecting first backscattered radiation through the first optical fiber and second backscattered radiation through the second optical fiber;
- producing a first signal based on the first backscattered radiation, and a second signal based on the second backscattered radiation;
- determine a measured differential backscatter signal as a function of wavelength using the first and second signals,

characterized by

- calculating the physical feature by curve fitting the measured differential backscatter signal to a backscatter function, in which the backscatter function is a function of a mean free path of photons. In this method, it is assumed that only singly scattered photons contribute to the differential backscatter signal and as a result the backscatter function can be easily derived analytically.

In an embodiment, the physical feature is a concentration of at least one substance in the medium.

The invention also relates to a device according to claim 13.

Furthermore, the invention relates to a computer program according to claim 14 and to a data carrier according to claim 15.

Finally, the invention relates to a method according to claim 16.

The present invention will be described below with reference to exemplary embodiments and the accompanying schematic drawings, in which:

Fig. 1 is a schematic diagram of a measuring device according to a preferred embodiment;

Fig. 2a and 2b show cross sections of a sample and two fiber tips in the situation wherein the mean free path of the photons is much larger than the diameter of the fibers;

Fig. 3 shows the results of Monte Carlo simulations for a homogeneous medium;

Fig. 4 a differential backscatter signal normalized at zero absorption for several scattering coefficients;

Fig. 5 shows a differential backscatter signal of a dilute suspension of 0.2 μm polystyrene spheres

Fig. 6 shows the total differential backscatter signal as a function of the reflection coefficient $\mu_s(\lambda)$ in the range of 10-100 mm^{-1};

Fig. 7 shows the measured and calculated average path length τ as a function of the average scattering coefficient;

Fig. 8 is a graph of measurement results for three different absorption coefficients μ_a showing the dc-fiber signal I, the c-fiber signal J and the differential backscatter signal R_{bs} as a function of wavelength;

Fig. 9 shows a typical spectrum of an absorption curve A along with the specific absorption coefficient of Evans Blue dye;

Fig. 10 shows a measured A^* as a function of the absorption coefficient μ_a at $\lambda = 600$ nm;

Fig. 11 shows typical spectra measured in a suspension of 1.0 μm polystyrene spheres with and without Evans Blue dye;

Fig. 12 graphically shows a molar extinction coefficient as a function of wavelength;

Fig. 13 shows in vivo measurements and a fit of the differential backscatter signal R_{bs} in a human trachea realized using a fiber diameter of 400 μm, and

Fig. 14 shows in vivo measurements and a fit of the differential backscatter signal R_{bs} in a human trachea showing very low oxygenation indicative for lung tumor.

A schematic diagram of a preferred embodiment according to the invention is shown in figure 1. The setup consists of a set of optical fibers for the delivery and collection of light to and from a sample 1 under investigation. Light from a light source 2, for example a Tungsten Halogen lamp (Avantes HL-2000-FHSA), is led through a

first arm 3 of a bifurcated optical fiber. The bifurcated optical fiber is at a distal end 4 coupled to a first distal end of a delivery- and-collection fiber 5 (in the following referred to as dc-fiber 5) which is small enough to be fit through a working channel of a clinical endoscope, not shown. A second distal end of the delivery- and-collection fiber 5 contacts the sample 1. Alongside the dc-fiber 5, a collection fiber 6 is arranged to collect light reflected by the sample 1. The collection fiber 6 (referred to as c-fiber 6) is connected to a slave channel of a dual-channel spectrometer 7, for example an Avantes SD2000. Preferably, the dc-fiber 5 is polished at a small angle to reduce specular reflections.

Light reflected back from the sample 1 into the c-fiber 6 is led directly into the slave channel of the dual-channel spectrometer 7. A second arm 8 of the bifurcated fiber is connected to a master channel of the dual-channel spectrometer 7. Light reflected into the dc-fiber 5 is coupled back into the bifurcated fiber, and reaches the dual-channel spectrometer 7 via the second arm 8 of the bifurcated fiber. An output of the spectrometer 7 is connected to an input of a processor 9 which is arranged to analyze signals from the spectrometer 7.

If only the dc-fiber 5 would be used to deliver and collect light to and from the sample 1, a large fraction of collected light is due to single backscattering from small sample depths, see [1]. A single to multiple scattering ratio depends on the scattering coefficient and phase function of the sample 1 and on a diameter of the dc-fiber 5. The contribution of multiply scattered light to the signal of the dc-fiber 5 can be approximately determined by combining the signal of the dc-fiber 5 with a signal coming from an additional fiber, i.e. the c-fiber 6 mentioned above.

In [4] a differential backscatter signal R_{bs} as a function of the wavelength λ is determined using a formula like

$$R_{bs}(\lambda) = c \cdot \left(\frac{(I(\lambda) - I_n(\lambda))}{(I_{white}(\lambda) - I_{black}(\lambda))} - \frac{J(\lambda)}{J_{white}(\lambda) - J_{black}(\lambda)} \right) \qquad (1)$$

where $I(\lambda)$ is the signal from the dc-fiber 5 in contact with the sample 1, $I_n(\lambda)$ is the signal from the dc-fiber 5 submersed in a fluid with an appropriate refractive index (for tissue: water would be appropriate), $I_{white}(\lambda)$ is the signal from the dc-fiber 5 with the

probe-tip at a specific distance from a diffuse reflecting reference material with a large, preferably wavelength-independent reflectance coefficient (white spectralon) and $I_{black}(\lambda)$ is the signal from the dc-fiber 5 with the probe-tip at that same specific distance from a diffuse reflecting reference material with a small, preferably wavelength-independent reflectance coefficient (black spectralon). Furthermore, $J(\lambda)$ is the signal from the c-fiber 6 in contact with the sample 1 and $J_{white/black}(\lambda)$ is the signal from the c-fiber 6 with the probe-tip at the previously mentioned specific distance from the white/black spectralon. Finally, c is a calibration constant that depends on the distance between the probe-tip and the reference materials.

According to the invention, the processor 9 is arranged to calculate the physical feature using a predefined mathematical model, the differential backscatter signal (R_{bs}) and a curve fitting mechanism. In an embodiment, the diameter of the fibers 5, 6 are selected depending on a mean free path (mfp) of photons sent into the sample 1. It is noted that if the mean free path can not be estimated before selecting a fiber diameter, initially two arbitrary fiber diameters may be selected. After curve fitting the measuring results using two different mathematical models, it will show which model applies.

Figures 2a and 2b depict fiber tips of the dc-fiber 5 and the c-fiber 6 in the situation wherein the mean free path (mfp) of photons coming out of the dc-fiber 5, is much larger than a diameter d_{fiber} of the fibers 5, 6. In an embodiment, the diameters of both fibers 5, 6 are of equal size, however it should be understood that other selections are possible. In figure 2a, lines 21 and 22 show an example of a path traveled by a detectable singly scattered photon. In figure 2b, lines 23, 24, 25 and lines 23, 24, 26 show two possible paths of detectable multiply scattered photons. All multiple scattering events occur at such large distances from the fiber tip of the fibers 5, 6, that the probability of detection of multiply scattered photons is roughly equal for the dc-fiber 5 and the c-fiber 6. The differential backscatter signal $R_{bs}(\lambda)$ will now purely be determined by singly scattered photons.

In an embodiment, the diameter of the fibers 5, 6 are selected so that mfp>d_{fiber}. In the predefined mathematical model of this embodiment, the differential backscatter signal $R_{bs}(\lambda)$ is an exponential function of two times the mean free path. Below, an explanation for this model is given.

In the absence of absorbers, the differential backscatter signal $R_{bs}(\lambda)$ is proportional to the local, superficial scattering coefficient $\mu_s(\lambda)=Q_{sca}(\lambda)\cdot\rho\cdot As$:

$$R_{bs}(\lambda)= C_{app}\cdot\frac{1}{4\pi}\cdot\int_{\Omega_{NA}} d\Omega\cdot p(\lambda,\Omega)\cdot Q_{sca}(\lambda)\cdot\rho\cdot As \qquad (2)$$

where C_{app} is an apparatus constant that depends amongst others on the distance between the probe tip and the reference materials (black and white spectralon), $p(\lambda,\Omega)$ is a function called the phase function where Ω is the scattering angle, $Q_{sca}(\lambda)$ the scattering efficiency, ρ the concentration of substances present in the sample 1, and As the area of a scattering particle. For example, using a fused silica fiber with numerical aperture NA= 0.22, the differential backscatter signal $R_{bs}(\lambda)$ can be approximated by

$$R_{bs}(\lambda)\approx C_{app}\cdot\frac{1}{4\pi}\cdot\int_{0}^{2\pi} d\varphi\cdot\int_{170}^{180} d\theta\cdot\sin(\theta)\cdot p(\lambda,180)\cdot\mu_s(\lambda) = C_{app}'\cdot p(\lambda,180)\cdot\mu_s(\lambda) \qquad (3)$$

where φ is the azimuthal angle and θ is the polar angle.

Figure 3 shows the differential backscatter signal $R_{bs}(\lambda)$ of measurements (see dots) of a dilute suspension of 0.2 μm polystyrene spheres along with a calculation (see curve 32) according to Eq. (3). In figure 3 $R_{bs}(\lambda)$ is shown using arbitrary units (a.u.). Also a value of Q_{radar} where $Q_{radar} = 4\pi\cdot p(\lambda,180)\cdot Q_{sca}(\lambda)$, is indicated in the figure. Figure 3 shows excellent agreement between the measurement (i.e. the dots) and the calculation, which indicates that if mfp>d_{fiber} , the single scattering is indeed the dominant contributor to the differential backscatter signal $R_{bs}(\lambda)$ as defined in Eq. (1).

A singly scattered photon first travels from the tip of the dc-fiber 5 to a particle, and then (the same distance) back from the particle to the tip of the dc-fiber 5 (or tip of c-fiber 6), see also figure 2a. So an average path length $\tau(\lambda)$ traveled by the measured single scattered photons is equal to two times the mean free path mfp(λ), i.e.

$$\tau(\lambda) = 2\cdot mfp(\lambda) \qquad (4)$$

In the presence of n absorbing species with specific absorption coefficients $\mu_a^{spec,i}(\lambda)$, the differential backscatter signal becomes

$$R_{bs}(\lambda) = C_{app}' \cdot p(\lambda,180) \cdot \mu_s(\lambda) \cdot \exp(-\tau(\lambda) \cdot \sum_{i=1}^{n} \rho_i \cdot \mu_a^{spec,i}(\lambda))$$

$$= C_{app}' \cdot p(\lambda,180) \cdot \mu_s(\lambda) \cdot \exp(-2 \cdot mfp(\lambda)) \cdot \sum_{i=1}^{n} \rho_i \cdot \mu_a^{spec,i}(\lambda)) \quad (5)$$

where C_{app}' is an apparatus constant, $p(\lambda,180)$ is the phase function, $\mu_s(\lambda)$ is the scattering coefficient of the medium, λ is the wavelength of the first and second backscattered radiation, mfp(λ) is the mean free path as a function of the wavelength, n is the number of substances in the sample 1, ρ_i is the concentration of absorber i present in a detection volume of the sample 1, and $\mu_a^{spec,i}(\lambda)$ is the absorption coefficient of absorber i as a function of the wavelength.

It is noted that in Eq. (5) the assumption is made that absorbers are homogeneously distributed and do not influence each other. The Eq. (5) may be corrected for non-linear phenomena such as an inhomogeneous distribution of absorbers, see e.g. [8].

According to an embodiment, the specific absorption coefficients of the absorbers, the wavelength dependency of the scattering coefficient μ_s and the phase function p, together with Eq. (5) are used in order to calculate the concentrations of all the absorbing substances present in the detection volume of the sample 1. Since the detection volume is typically very small in the present invention, the extracted concentrations are highly spatially resolved. This is not possible with the known methods that are based on diffuse reflectance, and wherein the obtained concentrations are averages over large sample volumes, see e.g. [2].

The apparatus constant C_{app}' (Eq. 3) can be determined for a specific distance between the tip of the dc-fiber 5 and the reference materials (black and white spectralon). For a suspension of monodisperse polystyrene spheres of known size and concentration, the scattering coefficient μ_s and the phase function $p(180)$ can be calculated using Mie theory [4]. The apparatus constant C_{app}' simply follows from Eq. (3). In terms of the volume fraction f of the suspension, the radius of the spheres a and

the radar efficiency coefficient $Q_{radar}(\lambda)= 4\pi \cdot p(\lambda ,180)\cdot Q_{sca}(\lambda)$ the apparatus constant is determined by

$$R_{bs}(\lambda) \approx C_{app}' \cdot p(\lambda,180)\cdot \mu_s(\lambda) = C_{app}' \cdot 0.05968 \cdot \frac{f}{a} \cdot Q_{radar}(\lambda) \qquad (6)$$

According to another embodiment, the selected diameter d_{fiber} is chosen so that the mean free path is smaller than d_{fiber}. In this embodiment, the differential backscatter signal R_{bs} is a function of the fiber diameter d_{fiber}. This will be discussed in more detail below.

When the mean free path of the photons is smaller than the selected fiber diameter (i.e. mfp$(\lambda)<d_{fiber}$), the contribution of multiply scattered light to the differential backscatter signal $R_{bs}(\lambda)$ of the single dc-fiber 5 cannot completely be removed using Eq. (1). In this case, it appears that the average path length of the photons contributing to the signal $R_{bs}(\lambda)$ becomes nearly independent of the optical properties of the sample 1. In this situation, multiple scattering events already occur at small distances from the tip of the dc-fiber 5. An analytical expression for the backscatter signal $R_{bs}(\lambda)$ is not available for this situation and Monte Carlo simulations were used to model the behaviour of $R_{bs}(\lambda)$ as a function of the diameter of the fibers 5, 6 and of the optical properties of the sample 1. Figure 4 shows the results of Monte Carlo simulations using the MCML-code (Monte Carlo for Multi-Layered media) of Wang et al [6,7] for a homogeneous medium with an anisotropy value g=0.9. A flat circular incident beam with diameter d_{fiber} is directed onto the sample 1, and the differential backscatter signal R_{bs} is calculated by subtracting the total reflectance in the c-fiber 6 (with diameter d_{fiber} and center located at a distance d_{fiber} from the center of the incident beam) from the total reflectance in the dc-fiber 5 (with diameter d_{fiber} overlapping the incident beam). Simulations were performed for sets of four different scattering coefficients (μ_s=15, 25, 50 and 80 mm^{-1}), four different fiber diameters (d_{fiber}=200, 400, 600 and 800 μm) and five different absorption coefficients (μ_a=0, 0.2, 0.4, 0.6 and 0.8 mm^{-1}).

Figure 4 shows R_{bs} as a function of absorption coefficient μ_a where the open circles/dashed lines correspond to d_{fiber}=200 μm, the filled circles/dotted lines correspond to d_{fiber}=400 μm, the open squares/solid lines correspond to d_{fiber}=600 μm

and the filled squares/dash-dotted lines correspond to d_{fiber}=800 μm. The differential backscatter signal R_{bs} for each scattering coefficient μ_s was normalized to the (d_{fiber}=200 μm, μ_a=0 mm^{-1}) case. Figure 4 shows that in the absence of absorption, i.e. $\mu_a = 0$, the differential backscatter signal R_{bs} depends linearly on the scattering coefficient μ_s. Furthermore, the slope of the straight lines (signifying the relation between R_{bs} and μ_a) depends only on the fiber diameter and is independent of the scattering coefficient μ_s. The latter is more clearly demonstrated in figure 5, where the differential backscatter signal R_{bs} is normalized to unity at zero absorption for all scattering coefficients μ_s. The open circles correspond to d_{fiber}=200 μm and the filled squares correspond to d_{fiber}=800 μm. These Monte Carlo simulations therefore suggest that in the situation where mfp<d_{fiber}, the diameter of the fibers 5, 6 determines the average path length τ of the measured photons. The backscatter signal R_{bs} for this range of parameters can thus be written as

$$R_{bs}(\lambda) = C_1 \cdot \mu_s \cdot \exp(-\tau \cdot \mu_a) = C_1 \cdot \mu_s \cdot \exp(-C_2 \cdot d_{fiber} \cdot \mu_a) \qquad (7)$$

where C_1 and C_2 are constants, τ is the average path length, μ_a is the absorption coefficient, μ_s is the scattering coefficient and d_{fiber} is the fiber diameter of the fibers 5, 6.

An exact analytical expression for $R_{bs}(\lambda)$ is not available due to the large contribution of multiple scattering events to the signal. Measurements were done for determining a total integrated backscatter signal R_{tot} for a range of λ between 400-900 nm, using the formula

$$R_{tot}(\mu_s) = \int_{400nm}^{900nm} d\lambda \, R_{bs}(\lambda, \mu_s) \qquad (8)$$

Figure 6 shows that the integrated total backscatter signal $R_{tot}(\mu_s)$ is proportional to $\mu_s(\lambda)$ in the relevant range of μ_s between 10-100 mm^{-1}. Therefore, in the absence of absorbers, it follows that

$$R_{bs}(\lambda) = C_{app} \cdot \mu_s(\lambda) \tag{9}$$

which is in agreement with the Monte Carlo simulations.

In the presence of n absorbing substances in a suspension, with specific absorption coefficients $\mu_a^{spec,i}(\lambda)$, the differential backscatter signal becomes

$$R_{bs}(\lambda) = C_{app} \cdot \mu_s(\lambda) \cdot \exp(-\tau \cdot \sum_{i=1}^{n} \rho_i \cdot \mu_a^{spec,i}(\lambda)) \tag{10}$$

where τ is the average path length of the detected backscattered photons and ρ_i is the concentration of the substance i.

Non-linear phenomena such as an inhomogeneous distribution of absorbers are not incorporated in Eq. (10), but can be added by the skilled person, see e.g. [8].

Figure 7 shows the measured and calculated average path length τ as a function of the average scattering coefficient $<\mu_s(\lambda)>$ (with 500 nm $< \lambda <$ 700 nm) for $d_{fiber} = 0.4$ mm and for absorption coefficient $\mu_a(\lambda) = 2.0$ mm^{-1} at λ=600 nm. In figure 7 measurement are indicated by dots, the $\tau = 2 \cdot$mfp curve is indicated by a line 71 and Monte Carlo simulations are depicted by dashed lines. Identical results were obtained for suspensions with an absorption coefficient of $\mu_a = 1.0$ mm^{-1} at 600 nm. The average path length τ was determined using suspensions of polystyrene spheres with different sizes and concentrations to vary the scattering coefficient $\mu_s(\lambda)$. The anisotropy g of these suspensions was in the range of 0.8-0.9. Evans Blue dye was added as an absorber, and the average path length τ was calculated from Eqs. (9) and (10) and knowledge of the concentrations and specific absorption coefficient of Evans Blue, as will be known to the skilled person.

Looking at the measured average path lengths of figure 7, it clearly shows that for large scattering coefficients (μ_s = 10-100 mm^{-1}, the range relevant for tissue) the average path length τ is independent of the scattering coefficient μ_s to within 10% and approximately equal to half the fiber diameter ($\tau \approx 0.24$ mm while $d_{fiber} = 0.40$ mm), in agreement with the Monte Carlo simulations (the dashed lines correspond to Monte

Carlo calculations for d_{fiber} = 0.2, 0.4, 0.6 and 0.8 mm). For small scattering coefficients (e.g. $\mu_s < 5$ mm^{-1}) the average path length τ is well described by $\tau = 2 \cdot$mfp, according to Eq.(4), see line 71. Figure 7 also clearly demonstrates that the transition from the 'single scattering regime' to the 'constant path length regime' occurs for mean free paths of the order of the fiber diameter. It is therefore expected that single scattering will prevail over a larger range of scattering coefficients for fiber diameters smaller than 400 μm.

In the following, the effect of absorption on the average path length τ will been examined in more detail. Various concentrations of Evans Blue dye were added to a suspension of polystyrene spheres with scattering coefficients μ_s of 35 mm^{-1}. The concentrations of Evans Blue (EB) dye were varied such that the absorption coefficient μ_a at 600 nm was in the range of 0 to 2 mm^{-1}. Typical results of the differential backscatter signal R_{bs} for three different absorption coefficients μ_a are shown in figure 8. Note that the signal I of the dc-fiber 5 is plotted on a different vertical scale than the signal J of the c-fiber 6 and the differential backscatter signal R_{bs}.

The spectra with Evans Blue R^{EB} present in the suspension were divided by the spectrum with no Evans Blue present R^0 and the negative natural logarithm of the ratio R^{EB}/R^0 was determined:

$$A = -\ln(R^{EB}/R^0) = \tau \cdot \rho \cdot \mu_a^{spec,EB} \qquad (11)$$

where ρ is the concentration of Evans Blue, and $\mu_a^{spec,EB}$ is the specific absorption coefficient of Evans Blue.

Figure 9 shows a typical spectrum of an absorption curve 92, along with the specific absorption coefficient $\mu_a^{spec,EB}$ of Evans Blue dye, see curve 94.

For all concentrations, an area A^* under the absorption curve 92 was determined in the wavelength range λ between 500 and 650 nm. From Eq. (11) it follows that if the average path length τ is independent of the absorption coefficient μ_a^{spec}, area A^* should depend linearly on the concentration ρ of the species in the suspension.

Figure 10 shows a measured A^* as a function of the absorption coefficient μ_a at 600 nm. A curve fitting is done, resulting in a line 104 for $\mu_s(\lambda) = 35$ mm^{-1}. Figure 10

shows that the average path length τ is indeed independent of the absorption coefficient μ_a in the range 0-2 mm^{-1}.

From the previous results of figure 4 to 10, it shows that for mfp < d_{fiber}, the differential backscatter signal R_{bs} is described by Eq. (7) with $C_2 \approx 0.6$. Figure 11 shows typical spectra measured in a suspension of 1.0 μm polystyrene spheres with and without Evans Blue dye ($\mu_a = 2$ and 0 mm^{-1} at 600 nm, respectively). From Eq. (7) and figure 7, the relation between the differential backscatter signals with and without absorber is given by

$$R_{bs}(\lambda,\mu_a) = R_{bs}(\lambda,0)\cdot exp(-0.24\cdot\mu_a) \qquad (12)$$

The calculated spectrum according to Eq. (12) is plotted as a dashed line 110 in figure 11 and shows excellent agreement with the measured $R_{bs}(\lambda,\mu_a)$, see line 111. In figure 11, line 112 depicts $R_{bs}(\lambda,0)$.

In short, the average path length τ of photons measured when subtracting the signals of the c-fiber 6 from the dc-fiber 5 using Eq. (1), is independent of the optical properties of the sample 1 and approximately equal to half the diameter of the fibers 5, 6 used, as long as the fiber diameter is larger than the mfp.

In a specific embodiment, the device according to the invention is arranged to determine concentrations of oxygenated blood in tissue. Since the scattering coefficient of tissue μ_s^{tissue} is in the range of 10-100 mm^{-1}, the fiber diameter should be smaller than a certain maximum diameter d_{max} where d_{max} is between 10 and 100 μm, so for example smaller than 50 nm, in order to measure predominantly single scattering in tissue. In this case, Eq. (5) holds. For fibers 5, 6 with much larger diameters (e.g. 200 or 400 μm), the differential backscatter signal $R_{bs}(\lambda)$ is described by Eq. (10) with $\tau \approx 0.6\cdot d_{fiber}$.

It is presently known that the wavelength dependence of the scattering coefficient in tissue μ_s^{tissue} can be adequately described by an empirical power-law function, see also [3], [4], [5].

$$\mu_s^{tissue}(\lambda) = a \cdot \lambda^{-b} \qquad (13)$$

with *a* and *b* constants that depend on the size, concentration and relative refractive index of the scatterers (i.e. substances) present in the detection volume.

The dominant absorbers in tissue in the visible wavelength range are oxygenated and deoxygenated blood. Thus in tissue Eq. (10) becomes

$$R_{bs}(\lambda) = C_{app} \cdot a\lambda^{-b} \cdot \exp(-0.6 \cdot d_{fiber} \cdot \rho_{blood} \cdot (S_{O2} \cdot \mu_a^{spec,ox} + (1-S_{O2}) \cdot \mu_a^{spec,deox}))$$

$$= C'_{app} \cdot \lambda^{-b} \cdot \exp(-0.6 \cdot d_{fiber} \cdot \rho_{blood} \cdot (S_{O2} \cdot \mu_a^{spec,ox} + (1-S_{O2}) \cdot \mu_a^{spec,deox})) \quad (14)$$

where ρ_{blood} is the concentration of blood, S_{O2} is the blood oxygenation (percentage oxygen saturation) in a certain detection volume, Capp is a constant that depends on the calibration constant *c*, C'app is $C_{app} \cdot a$, λ is the wavelength, b is the slope of the scattering coefficient defined in Eq. 13, $\mu_a^{spec,ox}$ is the specific absorption coefficients of fully oxygenated blood, $\mu_a^{spec,deox}$ is the specific absorption coefficients of fully deoxygenated blood.

Non-linear phenomena such as an inhomogeneous distribution of absorbers are not incorporated in Eq. (14), but can be added by the skilled person, see e.g. [8].

Since the specific absorption coefficients of fully oxygenated ($\mu_a^{spec,ox}$) and fully deoxygenated ($\mu_a^{spec,deox}$) blood are well known, see figure 12, Eq. (14) can be fitted to the measured data to yield the slope *b* of the scattering coefficient μ_s^{tissue}, the concentration ρ_{blood} and the oxygen saturation S_{O2} of the blood present in the detection volume. When a correction is made for the inhomogeneous distribution of blood in the vessels, a vessel diameter *D* may be determined as well. Since the average detection depth is small (e.g. 0.1 mm), the blood present in the detection volume when measuring non-invasively is located in capillaries.

In figure 13, in vivo measurements of backscattering in a human trachea together with a fit using Eq. (14) are shown. The measurements are realized using a fiber diameter of 400 μm. The dots depict the measurements and curve 130 is a fitting curve. In figure 13, $b = -0.94$ and the oxygenation $S_{O2} = 95\%$.

The present invention can be used for tumor detection. Tumor growth may, due to its excessive oxygen consumption, be accompanied by a low capillary oxygen saturation, which can only be assessed using a very localized measurement. Since (pre-

)cancerous tissue is generally more heterogeneous than normal tissue, the standard deviation of multiple measurements is likely to be larger for (pre-)cancerous tissue than for normal tissue. Standard deviations in the measurements can be calculated for the oxygen saturation, the blood concentration, the blood vessel diameter, and the slope b of the scattering coefficient μ_s^{tissue}. It is noted that the invention is by no means restricted to determine a concentration of a substance as the physical feature. All features, mentioned in the previous phrase can be regarded as physical features.

An example of a measurement of a lung tumor is shown in figure 14. The shape of the dip in the wavelength range of 500-600 nm in this figure demonstrates the depletion of oxygen from the capillaries of this tumor due to its excessive oxygen consumption.

When a needle-probe is used, the local oxygenation and scattering coefficient μ_s^{tissue} can be measured invasively. This could be helpful in determining tumor-margins intra-operatively in real-time, for instance during resection of a breast-tumor.

According to an embodiment, the device comprises multiple probes and a multichannel spectrometer for multiple simultaneous measurements on different locations of the sample 1. Using this device, multiple measurements can be made simultaneously on different locations of for example a suspicious lesion.

In yet another embodiment, the device comprises at least two pairs of fibers, having different fiber diameters. For example, when a pair of fibers with 100 μm, a pair of fibers with a diameter of 200 μm and a pair of fibers with a diameter of 400 μm are used, information from different depths in the sample 1 can be obtained as the average path length increases with increasing fiber diameter.

The method and apparatus according to the invention can also be used to analyze local drug concentrations. From Eq. (10) it follows that if the specific absorption coefficient of a certain drug is known, the local concentration ρ of that substance can be determined using the invention.

Another possibility of the present invention is to monitor glucose concentrations. The scattering coefficient μ_s^{tissue} depends among others on the relative refractive index of the scatterers with respect to the surrounding medium (in tissue: cytoplasm). The refractive index of the surrounding cytoplasm is likely to depend on the concentration

of glucose. A change in the glucose concentration will therefore likely affect the slope b of the scattering coefficient μ_s^{tissue}, see Eq. (13).

Whilst specific embodiments of the invention have been described above, it will be appreciated that the invention may be practiced otherwise than as described. For example, a concentration of a substance in polluted water may be calculated. The description is not intended to limit the scope of the invention.

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